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Short communication

Determination of mianserin in human plasma by high performance liquid chromatography–electrospray ionization mass spectrometry (HPLC–ESI/MS): Application to a bioequivalence study in Chinese volunteers

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ABSTRACT

This study aims to develop a standard protocol for the bioequivalence study of mianserin hydrochloride tablets—a tetracyclic antidepressant drug. For this purpose, a rapid, convenient and selective method using high performance liquid chromatography coupled with electrospray ionization mass spectrometry (HPLC–ESI/MS) has been developed and validated to determine mianserin in human plasma. Mianserin and the internal standard (I.S.), cinnarizine were extracted from plasma by *N*-hexane:dimethylcarbinol (98:2, v/v) after alkalinized with sodium hydroxide. LC separation was performed on a Thermo Hypersil-Hypurity C_{18} (5 μm, 150 mm × 2.1 mm) with the mobile phase consisting of 10 mM ammonium acetate (pH 3.4)–methanol–acetonitrile (35:50:15, v/v/v) at 0.22 ml/min. The retention time of mianserin and cinnarizine was 3.4 and 2.1 min, respectively. Quadrupole MS detection and quantitation was done by monitoring at *m/z* 265 $[M+H]^+$ for mianserin and *m/z* 369 $[M+H]^+$ for cinnarizine. The method was validated over the concentration ranges of 1.0–200.0 ng/ml for mianserin. The recovery was 81.3–84.1%, intra- and inter-day precision of the assay at three concentrations were 9.6–11.4% with accuracy of 97.5–101.2% and the lower limit of quantitation (LLOQ) detection was 1.0 ng/ml for mianserin. The stability of compounds was established in a battery of stability studies, i.e., short-term and long-term storage stability as well as freeze–thaw cycles. This method proved to be suitable for the bioequivalence study of mianserin hydrochloride tablets in healthy human male volunteers.

1. Introduction

Mianserin (1,2,3,4,10,14-*b*-hexahydro-2-methyl dibenzo [*c*,*f*] pyrazino [1,2-α] azepine) is a tetracyclic antidepressant drug, which blocks presynaptic alpha-2 receptors in the brain, resulting in higher levels of noradrenalin in the synaptic cleft, in combination with anti-histaminic properties and is used to treat depression and sleep disturbances [1,2]. It also has a peripheral serotonin antagonist effect [3]. It is structurally similar to mirtazapine, whose chemical structures are illustrated in Fig. 1. It is administered as a racemate of *R*- (−) and *S* (+)-mianserin hydrochloride in a dose of 30–90 mg/day. It takes 2–3 h to reach the peak concentration of mianserin after oral administration, the elimination half-life is 14–33 h and prolonged in the elderly [1].

Several methods are reported to detect mianserin by using HPLC [2,4–6], GC–MS [7–11] and capillary zone electrophoresis [12]. However, these methods present some disadvantages such as low sensitivity, poor reproducibility, time consuming or costly, i.e. derivatization step and long chromatographic run times. During the recent years, mass spectrometry has repeatedly been proven to be a powerful technique for the rapid, quantitative determination of drugs and metabolites in physiologic fluids. Syage and coworkers have reported the utilization of LC/MS and LC–MS/MS techniques for the determination of mianserin [13–16]. However, due to lacking the sample preparation details [13], time consuming in extraction procedures [14] (vortex three times for 2 min and centrifuge twice for another 20 min), low sensitivity [15] (LLOQ 0.1 μg/ml), requirement of special testing equipment, such as tandem mass spectrometry [16], and a large aliquots of sample was needed [14–16], these methods could not be used easily in most laboratories to analyze the samples in batches. In this study, a simple, rapid, and selective HPLC–ESI/MS method was developed and validated to determine mianserin in human plasma, and a stan-

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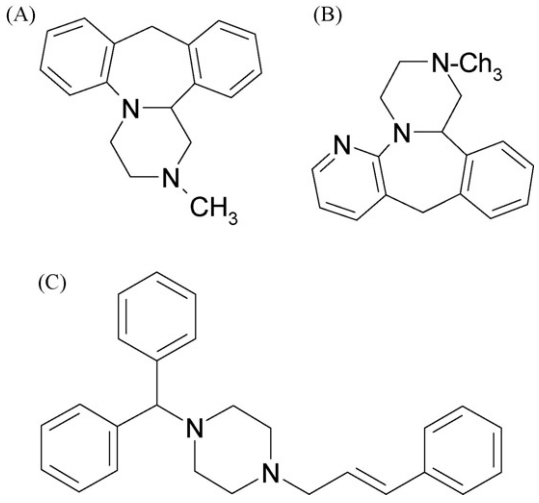

Fig. 1. Chemical structures of mianserin (A), mirtazapine (B) and cinnarizine (C).

dard protocol for bioequivalence study of mianserin hydrochloride tablets was devised.

2. Experimental

2.1. Materials and reagents

Mianserin hydrochloride standard (99.80%, lot: 301920) was kindly supplied by Vickmans Laboratories Limited, Hong Kong, China. Cinnarizine standard (99.53%) was purchased from Chengdu Great Southwest Pharmaceutical company Ltd. Ultra-pure water prepared by a Millipore Milli-Q purification system (Millipore Corp. Bedford, MA, USA) was used as the mobile phase. Acetonitrile and methanol were HPLC grade, and all other chemicals and solvents were of the highest analytical grade available. Drug-free and drug-containing plasma were taken from the volunteers. Plasma was stored at −20 °C until further use for analysis.

2.2. Instrumentation

A Shimadzu LC–MS 2010 system (Shimadzu, Kyoto, Japan) was used, equipped with LC-10AD VP low pressure gradient pump, CTO-10A VP column temperature oven, SCL-10AD VP system controller, and LC–MS chemstation (version 2.04). Separation was achieved on a Thermo Hypersil-Hypurity C_{18} column (150 mm × 2.1 mm, i.d., 5 μm) at 45 °C. Compounds were eluted up to a total retention time of 4 min using an isocratic mobile phase consisting of 10 mM ammonium acetate (pH 3.4)–methanol–acetonitrile (35:50:15, v/v/v) at a flow-rate of 0.22 ml/min, and the injection volume was 5 μl. The operating parameters of electrospray ionization-mass spectrometry (ESI–MS) were as follows: the mass resolution after calibration was 2 M; capillary voltage was 4.5 kV; cone voltage was 25 V; nebulizer nitrogen gas flow-rate was 2.5 l/min; drying N_2 flow was 10 l/min; drying gas temperature was 250 °C; detector voltage was 1.7 kV and the SIM dwell time was 100 ms. The mass selective detector (MSD) was operated in the positive ionization mode with selected-ion monitoring (SIM) at m/z 265 $[M+H]^+$ for mianserin and m/z 369 $[M+H]^+$ for cinnarizine.

2.3. Calibration standards and control samples

Primary stock solutions of mianserin (100.0 μg/ml) and cinnarizine (I.S. 100.0 μg/ml) were prepared in methanol and all the stock solutions were stored at 4 °C without any appreciable degradation for 4 weeks. Working solutions were prepared from the stock solutions by a series dilution. Routine daily calibration curves and quality control samples were prepared in drug-free plasma. The calibration standards were made to yield concentrations of 1.0, 2.0, 5.0, 10.0, 25.0, 50.0, 100.0 and 200.0 ng/ml. For the preparation of quality control samples, an independent stock solution was prepared and further diluted, to achieve concentrations of 2.0, 25.0 and 100.0 ng/ml, respectively.

2.4. Validation of the method

Analytical method validation was performed in accordance to the recommendations published by the FDA [17].

Specificity was ascertained by analyzing six blank human plasma samples without I.S. to determine the interference with mianserin. Matrix effects for mianserin were evaluated by comparing the peak areas of mianserin in extracted samples of blank plasma from six different drug-free volunteers spiked with known concentrations with the corresponding peak areas obtained by direct injection of standard solutions. Matrix effects for the I.S. were also investigated. The lowest standard on the calibration curve should be accepted as the limit of quantification if the following conditions are met. The analyte response at the lowest limit of quantitation should be at least five times the response compared to blank response. Analyte peak (response) should be identifiable, discrete, and reproducible with a precision of 20% and accuracy of 80–120%. The limit of detection (LOD) was the concentration with signal-to-noise ratio of 3. Six sets of calibration curves ranging from 1.0 to 200.0 ng/ml for mianserin were constructed. ANOVA was used to assess the results of the linear regression, and t-test was used for hypothesis testing in interval estimation of the regression parameters. The extraction recoveries were determined at three concentration levels by comparing peak area of the analytes obtained from plasma samples with the analytes spiked before extraction to those spiked after the extraction. Precision assays were carried out five times using three different concentrations (2, 25 and 100 ng/ml) on the same day and over five different days.

2.5. Sample preparation

50-μl aliquots of cinnarizine (I.S. 62.5 ng/ml) standard solution were added to 200 μl of each plasma sample and vortex-mixed. The plasma was then made alkaline by adding 50 μl 1 M sodium hydroxide solution. After a thorough vortex mixing for 20 s, the mixture was extracted with 1 ml of mixture of *N*-hexane:dimethylcarbinol (98:2, v/v), vortex-mixed for 3 min, and centrifuged at 14,000 rpm for 5 min at room temperature. The organic layer was removed and evaporated under a gentle stream of nitrogen gas at 40 °C. The dried residue was dissolved with 200 μl mobile phase. After centrifugation, 5 μl of the clear supernatant was injected into the LC–MS system.

2.6. Application of the assay

The method described in this paper was applied to a bioequivalence study of two oral formulations of mianserin hydrochloride (test formulation: Vick-Mianserin Tab®, 30 mg/tab, Lot: T0369, manufactured by Vickmans Laboratories Limited; reference formulation: Tolvon Tab®, 30 mg/tab, Lot: 743195, manufactured by N.V. Organon Oss Holland). The study followed a single dose, two-way randomized crossover design with a 2-week washout period between doses. The values of C_{max} (peak plasma concentration) and t_{max} (time to reach C_{max}) were calculated from the observed

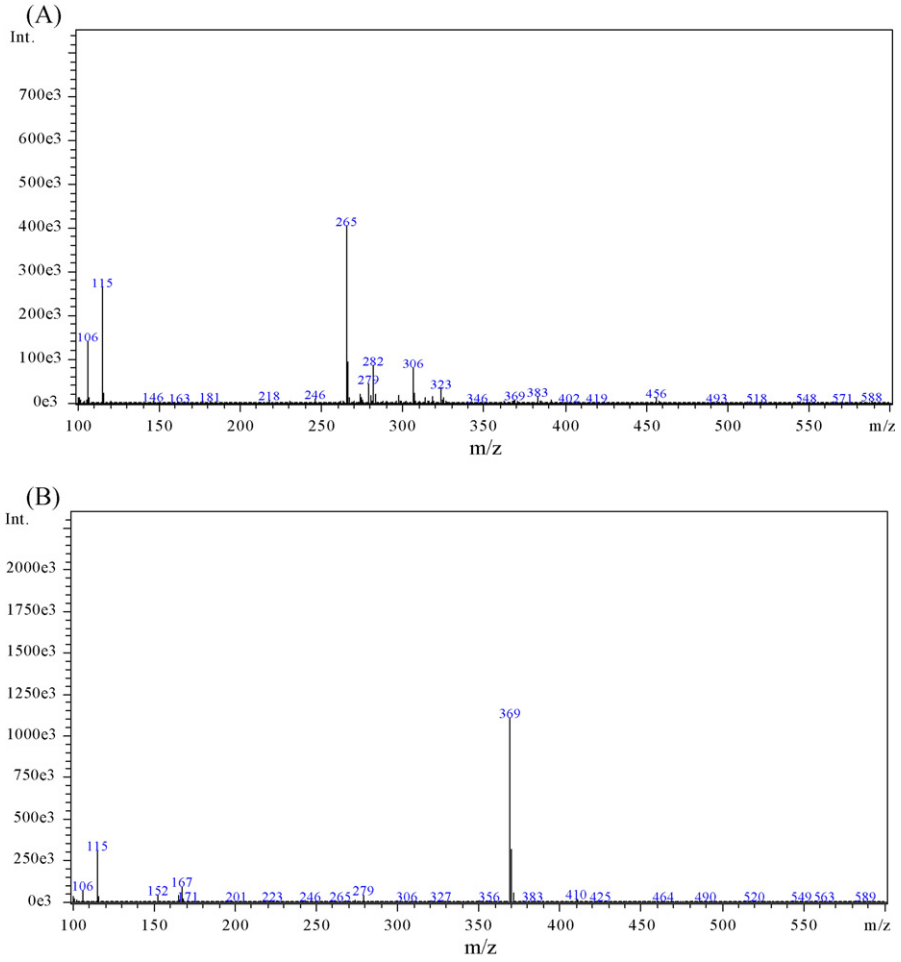

Fig. 2. Full scan mass spectra of mianserin (A) and cinnarizine (B).

parameters. The other major parameters including AUC (the area under the plasma concentration–time curve), and $t_{1/2}$ (elimination half-life) of the test and reference mianserin hydrochloride were calculated using one compartmental model by Drug and Statistics Software (DAS, version 2.0, China). ANOVA and *t*-test were used to compare the log-transformed C_{max} and AUC; a non-parametric test was used to compare t_{max} for the two formulations.

3. Results and discussion

3.1. HPLC–ESI/MS

The HPLC–ESI/MS in the SIR mode provided a highly selective method for the determination of mianserin and cinnarizine, the internal standard. The retention times were approximate 3.4 (3.405–3.460) and 2.1 (2.112–2.141) min, respectively. Compared with the published methods [4–13,15,16], the chromatographic run of this method was shortened, the complete elution was obtained in less than 4.0 min; the procedure was simple and just 0.2 ml plasma was needed. In our assessment of different mobile phase, 10 mmol/l ammonium acetate (pH 3.4), methanol and acetonitrile (35:50:15, v/v/v) provided the best effect which can eliminate the baseline noise completely during the chromatographic analysis. The alkalinized samples with sodium hydroxide improved extraction recovery and ammonium acetate was added in mobile phase for achieving good chromatographic peak shape and resolution. In general, ESI produced greater sensitivity and exhibited less interference than atmospheric pressure chemical ionization (APCI) sources. ESI positive MS spectra for mianserin and cinnarizine were dominated by the $[M+H]^+$ ions, i.e., *m/z* 265 $[M+H]^+$ for mianserin and *m/z* 369 $[M+H]^+$ for cinnarizine. Full scan ESI^+ mass spectra of standards, the chromatograms of blank human plasma, standards in blank human plasma and samples were shown in Figs. 2 and 3, respectively. In this study, we utilized a simple one-step extraction method. Among the various extraction solvents, we found that using *N*-hexane:dimethylcarbinol (98:2, v/v) can significantly improve the extraction efficiency and only 1 ml of the mixed liquor was needed. As demonstrated in this assay, this method is rapid, convenient, sensitive and specific for analyzing samples in batches and perfectly suitable for a high-throughput routine such as pharmacokinetic studies.

3.2. Validation of the method

3.2.1. Specificity

No significant interfering peaks were observed at the retention times of mianserin and cinnarizine in blank plasma extraction which are shown in Fig. 3A. In the present study, no matrix components in plasma caused significant changes in the MS response of mianserin. At mianserin concentrations ranging from 1 to 100 ng/ml, the mean peak area ratios were 0.96–1.02%; it was 1.01% for the I.S. In all cases, the R.S.D. values were below 8.1%. Thus, no matrix effect was observed.

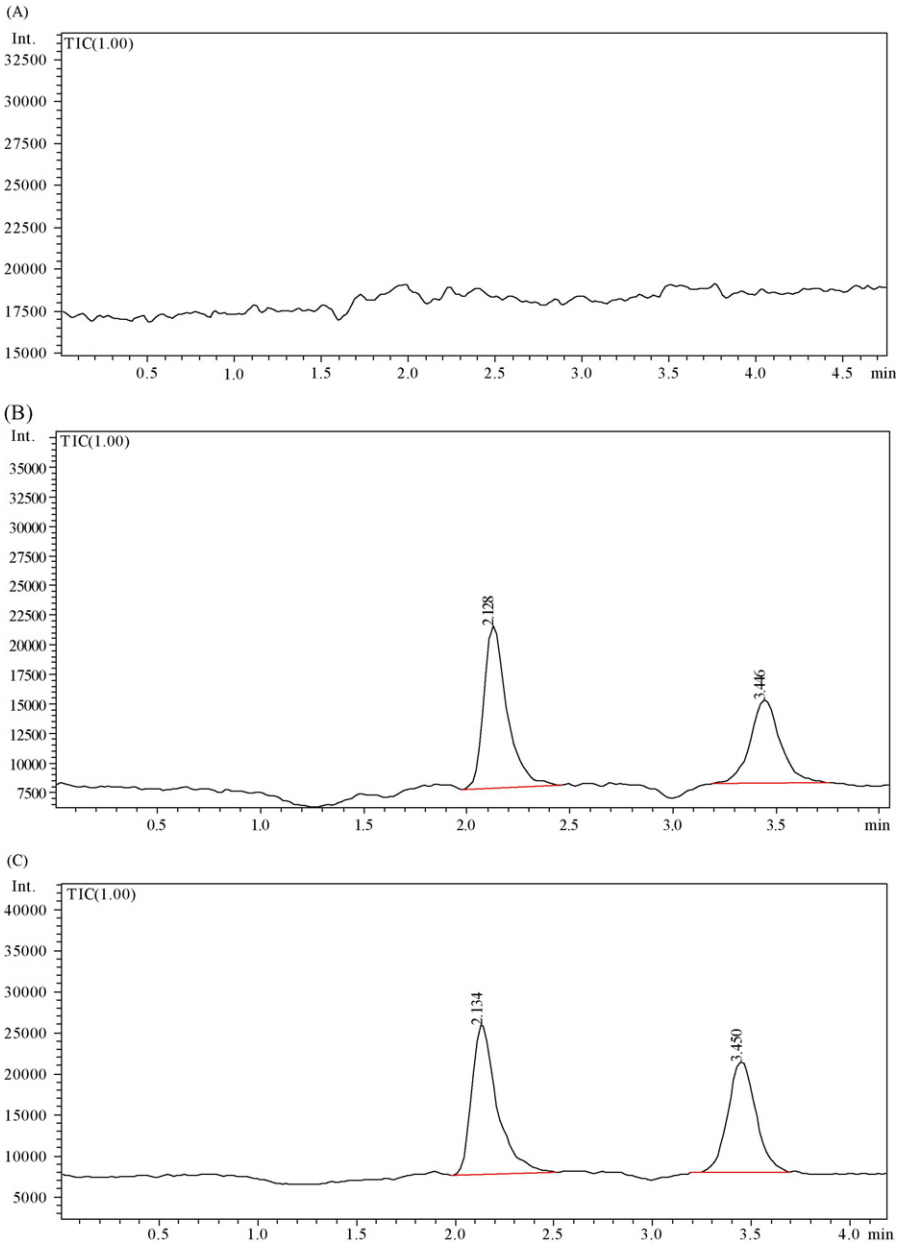

Fig. 3. Total ion chromatograms (TICs) of (A) blank plasma, and (B) plasma spiked with mianserin at 48.7 ng/ml and I.S. at 62.5 ng/ml, and (C) a plasma sample 1.5 h after single dose of mianserin hydrochloride tablet (60 mg, 62.3 ng/ml) in healthy volunteer. The retention time of mianserin and cinnarizine (I.S.) was 3.4 and 2.1 min, respectively.

3.2.2. Linearity and limit of quantitation

The calibration curve of mianserin was linear over 1.0–200.0 ng/ml. The equation was $y = 0.0459x + 0.0598$ ($r^2 = 0.9979$). The lower limit of quantitation (LLOQ) validated was 1.0 ng/ml (S/N = 5, Fig. 4) and the assay only required 0.2 ml of plasma.

3.2.3. Recovery, precision, accuracy and stability

The extraction recoveries, precision and accuracy of the method were summarized in Table 1. The extraction recovery of the internal standard is 74.5%. Stability quality control plasma samples (2.0, 25.0 and 100.0 ng/ml) were found stable in plasma when placed in the short-term (24 h) room temperature, three freeze/thaw (−20 to −25 °C) cycles and stored at −20 °C for 60 days (Table 2).

3.3. Bioequivalence study

This method was successfully applied to a bioequivalence study of two formulations of mianserin hydrochloride tablet in 12 healthy Chinese male volunteers, which was approved by the Ethical Committee of the Second Xiangya Hospital of Central South University. The subjects possessed good health and have not taken any medication for at least 2 weeks prior to the study. All subjects provided written informed consent prior to participating in the study. After administrated a single dose of mianserin hydrochloride (60 mg of either test or reference formulation), 4 ml of blood samples were taken before and 0.5, 1, 1.5, 2, 3, 4, 8, 12, 24, 48, 72, 96 and 120 h after dosing. After a washout period of 14 days, the study was repeated in the same manner to complete the crossover design. The mean plasma concentration–time profiles of mianserin after a single dose

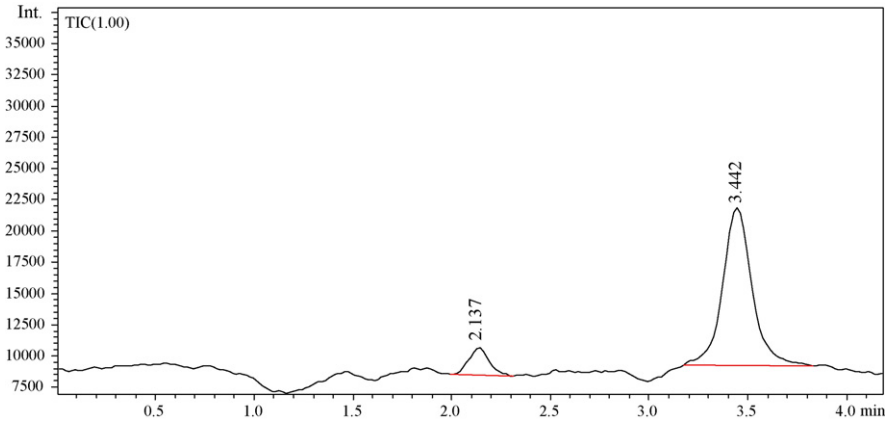

Fig. 4. A chromatogram at the LLOQ of mianserin (1.0 ng/ml).

Table 1
Recovery, inter- and intra-day precision and accuracy of mianserin

Concentration (ng/ml)	Recovery ($n=5$)		Intra-day ($n=5$)		Inter-day ($n=15$)	
	Mean (%)	CV%	Precision (R.S.D.%)	Accuracy (%)	Precision (R.S.D.%)	Accuracy (%)
2.0	81.3	7.9	9.8	101.0	10.5	100.7
25.0	84.1	8.5	10.2	97.5	11.4	98.0
100.0	82.5	6.9	9.6	101.2	10.0	100.7

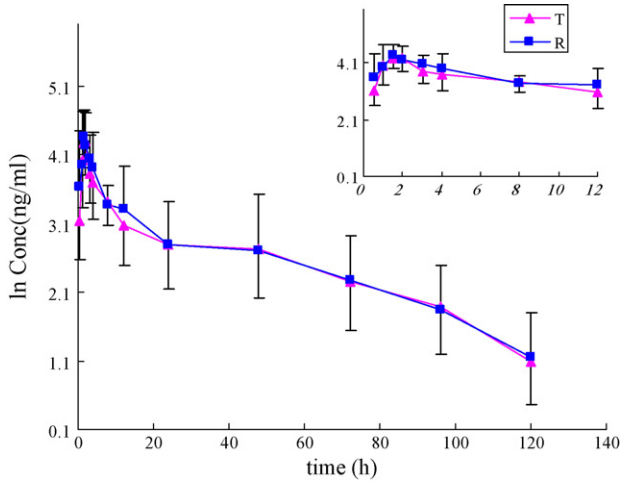

Fig. 5. Mean plasma concentration *vs.* time curve of mianserin after the administration of 60 mg test (Vick-Mianserin®; ▲) and reference (Tolvon Tablets®; ■) formulations to healthy Chinese male subjects. The inset figure is a zoom in the region between 0 and 12 h.

of 60 mg of either formulation were shown in Fig. 5. The pharmacokinetic parameters of the two mianserin formulations were shown in Table 3. The means and standard deviations of AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} for the test and reference formulation were similar, indicating that the pharmacokinetics of mianserin in the two formulations was similar. The 90.0% confidence intervals for the ratios of test drug to reference drug in terms of AUC_{0-t} and C_{max}, were within the range 80.0–125.0%, which is the range accepted by the State Food and Drug Administration [18].

Table 2
Stability data for mianserin

Concentration (ng/ml)	RT ($n=5$)		−20 °C ($n=5$)		Freeze/thaw ($n=5$)	
	Mean	R.S.D.(%)	Mean	R.S.D.(%)	Mean	R.S.D.(%)
2.0	2.16	9.0	2.0	7.8	1.9	11.3
25.0	24.4	6.5	25.5	9.3	24.7	5.9
100.0	101.2	8.7	99.7	6.4	101.1	7.4

RT: room temperature.

4. Conclusions

The purpose of the present study was to develop a standard protocol for the bioequivalence testing of mianserin hydrochloride tablet. We devised and validated, a rapid and convenient HPLC–ESI/MS method using a simple liquid–liquid extraction procedure and isocratic chromatography, to determine mianserin levels in human plasma, and used this method to conduct a bioequivalence study by administering 60 mg of mianserin hydrochloride tablets to healthy Chinese male volunteers. The

Table 3
Pharmacokinetic parameters (mean ± S.D.) of mianserin, after the administration of an oral dose of 60 mg Vick-Mianserin Tablets® and the reference drug (Tolvon Tablets®) formulations to healthy Chinese male volunteers

Parameters	Vick-mianserin Tab (T)	Tolvon Tab (R)	90.0% CI
t_{max} (h)	1.6 ± 0.4	1.8 ± 0.9	–
C_{max} (ng/ml)	102.3 ± 35.8	97.1 ± 35.8	96.5–118.1
AUC_{0-120} (ng h/ml)	1975.7 ± 927.2	2079.6 ± 921.3	85.6–107.6
$AUC_{0-\infty}$ (ng h/ml)	2172.7 ± 1019.2	2308.0 ± 1035.4	90.4–113.4
$t_{1/2}$ (h)	33.8 ± 9.6	35.2 ± 9.8	–

t_{max}: time to reach C_{max}; C_{max}: peak plasma concentration; AUC_{0-120}: area under the curve from 0 to 120 h; $AUC_{0-\infty}$: area under the curve from 0 to infinity; $t_{1/2}$: elimination half-life.

developed assay showed acceptable precision, accuracy, linearity, stability, and specificity. It can be easily repeated by most laboratories.

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